THE INCIDENCE OF DDS RESISTANCE IN LEPROMATOUS PATIENTS IN COSTA RICA: THEIR METABOLIC DISPOSITION OF DDS

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Costa Rica is a small country of about 1.7 million people. It has a relatively prosperous and broadly based economy, and there is little emigration. The incidence of leprosy is low (on the order of 30 new patients a year) and the follow-up of the patients is unusually complete. About half the patients are lepromatous and they are followed for life. When sulfone therapy began in 1945 the chief drugs used were Promin, Diasone, and other conjugated sulfones; since 1960, the chief drug has been dapsone (DDS).

The clinical records are complete and convenient, and they were relatively easy to search for patients with relapse possibly due to DDS-resistant M. leprae. In March 1973, the records of all patients admitted more than 7 years earlier were inspected and those patients who still had positive skin smears after 7 years of therapy were examined in more detail (upper half of Table 1). There were 16 patients who had definite increases in the number of bacteria from their skin smears in spite of apparently adequate therapy, and 8 patients who had definite increases during apparently inadequate therapy. In addition, there was one patient with a probable increase in bacteria during apparently adequate therapy.

TABLE 1. COSTA RICAN PATIENTS STUDIED

Category	Increase in BI	Sulfone Therapy	No. of Patients
A	Definite	Adequate	16
B	Definite	Inadequate	8
C	Probable	Adequate	1

Category and No. of Patients Biopsied	Results of Mouse Inoculation				
	No Growth	Sensitive to .0001 g% DDS	Resistant to DDS		
			.0001 g%	.001 g%	.01 g%
A, 14	1	3	3[a]	2	5[b]
B, 4	3				1
C, 1			1		
Total	7		12		

[a] Results at .001 and .01 g% DDS not available for one strain.

[b] Two strains were partially resistant to .01 g% DDS.

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As shown in the lower half of Table 1, biopsy specimens for inoculation of mice were obtained from 14 of the patients in category A. No growth in mice is attributed to favorable response of the bacilli to drug in the patient's tissues and thus one patient of category A is considered to have DDS-sensitive M. leprae. DDS-sensitive strains were isolated from 3 patients and DDS-resistant strains from 10. Two patients in category A refused biopsy. One had shown a definite increase in bacteria during DDS therapy and a decrease following subsequent therapy with clofazimine; the other showed further deterioration between March 1973 and March 1975. Thus, we concluded that these two patients had DDS-resistant disease.

Biopsy specimens were obtained from 4 of the 8 patients in category B: Three isolates showed no growth in the mice; one was found to be DDS-resistant. The one patient of category C provided an isolate that was DDS-resistant.

In summary, 7 patients were considered to have DDS-sensitive infections and 12 were DDS-resistant. The two patients of category A who refused biopsy were judged from later observations to have DDS-resistant infections, yielding a total of 14 DDS-resistant cases.

Table 2 shows that these 14 patients were admitted between 1941 and 1955. The time from the start of therapy to relapse ranged from 14 to 27 years; the time from the start of sulfone therapy to relapse was 10 to 24 years. In both ranges, some of the patients did not receive therapy for several years. The numbers in parentheses indicate the two patients who refused biopsy. As shown, the DDS-resistant patients represented 4.6% of all lepromatous patients admitted from 1941 to 1955, and 6.8% of the lepromatous patients admitted in those years and still living in 1973.

Thus, DDS-resistance can be considered a serious problem in Costa Rica but not an overwhelming one. It demands immediate attention, however, because the DDS-resistant strains must be considered to be fully infectious. The percentage incidence of relapse due to sulfone-resistant M. leprae in Costa Rica is lower than that observed by Meade and colleagues (1) for patients in Malaysia who started treatment on sulphetrone and also lower than that reported by Jacobson (2) for patients at the PHS Hospital at Carville, in the United States. Exact comparison with the cited reports is difficult, however, because of uncertainty about the clinical classification of the patients when they started sulfone therapy. All patients began sulfone therapy long before the Ridley-Jopling classification came into the picture and it is difficult now to know just what proportion of the patients in any of these studies would fall into BL, BL/LL, or LL groups, as recognized today.

Table 2

CHARACTERISTICS OF 14 PATIENTS WITH DDS-RESISTANT M. LEPRAE

Year of admission:	'41, '46, ('47), '48, '49, '49, '49, '50, '50, '51, '53, '54, '54, ('55)
Years from start of therapy to bacterial relapse:	14, 16, 16, 17, (18), 19, 19, 20, 20, 21, 22, (23), 26, 27
Years of sulfone therapy before bacterial relapse:	10, 12, 13, 13, 14, 16, (17), 18, 18, 19, 19, 21, (22), 24
Proportion of DDS-resistant patients in total LL patients (1941-1955):	14/303 = 4.6%
Proportion of DDS-resistant patients in total LL patients living in 1973:	14/205 = 6.8%

Previously (3), we reported that leprosy patients harboring DDS-resistant M. leprae exhibited an abnormal distribution of acetylator phenotypes and significantly faster plasma clearance rates of DDS than other groups of subjects. A recognized deficiency in those earlier studies was that the DDS-resistant group was comprised of patients of varied ethnic and racial origins and no matched control group was available for comparison. In contrast to the above, Ellard et al. (4) did not observe an unusual distribution of acetylator phenotypes in Chinese patients with DDS-resistant leprosy.

To examine more rigorously this problem of the metabolic characteristics of DDS-resistant patients, we studied the Costa Rican patients described above. The first phase of the current work involved the study of 21 lepromatous patients who had relapsed. This phase was completed before the results of tests of the susceptibility of their M. leprae in mice were available.

The pharmacologic testing involved determinations of the acetylator phenotype with sulfamethazine (SMZ) and the disposition of DDS in patients as outlined in Table 3.

Table 3

PROTOCOL FOR STUDIES IN COSTA RICAN PATIENTS

Pretreatment:
Patients withdrawn from all chemotherapy 5 days before study.
DDS test, Day 1:
50 mg DDS administered orally; heparinized blood sample taken 8 hr after DDS.
DDS test, Day 2:
Blood samples taken at 24 and 32 hr after DDS.
DDS test, Day 3; SMZ test:
Blood sample taken at 48 hr after DDS. Oral SMZ (10 mg/kg) given: urine collected during 0-6 hr; blood sample taken at 6 hr after SMZ.

Pretreatment: It was requested that patients be withdrawn from all drugs, including aspirin, but excepting antiovulatory drugs, five days before the study.

DDS Test, Day 1: Orally administer 50 mg DDS in early a.m. and collect 10 ml heparinized blood 8 hr after administration. After mixing and centrifugation, the plasma was separated and stored frozen.

DDS Test, Day 2: Obtain plasma samples as above, at 24 and 32 hr after the DDS administration on Day 1, and store them frozen.

DDS Test, Day 3; SMZ Test: Obtain plasma sample, as above, 48 hr after DDS administration on Day 1, and store frozen. Immediately thereafter, orally administer 10 mg SMZ/kg in water. Collect urine during 6 hr after the dose of SMZ. Measure total urine volume and record. Store a 50-ml sample frozen. At 6 hr after SMZ administration, collect a plasma sample as above and store frozen. Specimens were shipped frozen via air to our California laboratories.

Analyses for SMZ and acetyl SMZ (AcSMZ) in plasma and urine samples were performed by procedures described previously (5). DDS and monoacetyl dapsone (MADDS) levels in plasma were determined by recent modifications of our column chromatographic-fluorometric methods described earlier (6).

As shown previously in Table 1, the mouse foot pad inoculations showed that 7 of the non-responding patients were judged to have DDS-sensitive M. leprae and 12 to have DDS-resistant disease. For comparison, we subsequently studied 20 lepromatous Costa Rican patients whose disease was responding to DDS therapy.

The physical characteristics of all patients studied are shown in Table 4. Listed first is the control group of patients responsive to DDS therapy. Those who had relapsed are designated non-responders; they were subsequently subdivided into the 12 with DDS-resistant and the 7 with DDS-sensitive infections. Two of the original group of non-responders did not provide biopsy specimens. No significant differences were found in the distribution of sexes among the groups by the chi-square test using 2 × 2 contingency tables (7). As indicated, only minor differences were noted in either the mean age or the mean body weight of the various groups.

Table 4

PHYSICAL CHARACTERISTICS OF THE COSTA RICAN PATIENTS

Parameter	Responders	Non-Responders		
		Total	DDS-Resistant	DDS-Sensitive
Number	20	21	12	7
Sex distribution	4F; 16M	8F; 13M	6F; 6M	2F; 5M
Age (yr)[a]	58 ± 3	49 ± 2[b]	51 ± 4	44 ± 2[b]
Body weight (kg)[a]	64 ± 3	55 ± 2[b]	55 ± 4	55 ± 2

[a]Values are means ± SE.

[b]Significantly lower ($P < 0.05$) than the mean of the responders.

The second column of Table 5 presents the results of phenotyping with SMZ. From the percentage acetylation of this drug in plasma collected 6 hr after administration, each group was readily differentiated into rapid and slow acetylators as shown. There was no overlap in the values for the two phenotypes and each test of percentage acetylation

of SMZ in the 6-hr urine collection confirmed the results found in plasma. For brevity, the urinary results have not been included. The last column shows the combined mean acetylation of DDS at 8, 24, 32, and 48 hr after 50 mg DDS in these patients. As shown, these mean values also differentiated the rapid from the slow acetylators, although not so dramatically as did the SMZ test. All means for rapid acetylators were significantly different from those for slow acetylators. Using the chi-square test, we did not find a significant difference in the distribution of acetylator phenotypes among the responders, non-responders, DDS-resistant and DDS-sensitive groups.

Table 5

CHARACTERISTICS OF ACETYLATION OF SMZ AND DDS IN COSTA RICAN PATIENTS

Patient Group	% Acetylation in Plasma[a]	
	of SMZ	of DDS
Responders (20):		
Rapid acetylators (11)	76 ± 3	38 ± 3
Slow acetylators (9)	27 ± 3	21 ± 3
Non-responders (21):		
Rapid acetylators (14)	72 ± 2	37 ± 2
Slow acetylators (7)	29 ± 2	17 ± 1
DDS-resistant (12):		
Rapid acetylators (8)	74 ± 2	39 ± 2
Slow acetylators (4)	31 ± 1	18 ± 2
DDS-sensitive (7)		
Rapid acetylators (4)	68 ± 2	32 ± 4
Slow acetylators (3)	28 ± 4	16 ± 1

[a]Values are means ± SE.

Other characteristics of DDS disposition are shown in Table 6. In this table, the phenotypes of the various groups are not listed separately because phenotype had no influence on the parameters listed in this table. Because each patient received 50 mg DDS regardless of body weight, we have calculated weight-adjusted DDS plasma levels for comparing levels in the various groups. No significant differences were

noted between the mean levels shown for any combination of groups at 8, 24, or 48 hr after DDS. The same results were obtained from evaluations of the 32-hr plasma levels, which are not shown. In addition, no consistent differences were noted in the mean rates of clearance ($T_{\frac{1}{2}}$) of either DDS or MADDS from the plasma in any of the groups. The slightly longer $T_{\frac{1}{2}}$ value for MADDS in the total non-responders versus the responders we do not consider biologically significant. These results indicate that the total non-responding group, or its subgroups of DDS-resistant and DDS-sensitive patients, were not different from the patients responding to DDS therapy.

Table 6

CHARACTERISTICS OF DDS DISPOSITION IN THE COSTA RICAN PATIENTS

Parameter	Responders[a]	Non-Responders[a]		
		Total	DDS-Resistant	DDS-Sensitive
Number	20	21	12	7
Weight-adjusted DDS plasma levels (μg/ml ÷ mg/kg)				
at 8 hr	0.65 ± 0.03	0.60 ± 0.03	0.63 ± 0.04	0.56 ± 0.06
at 24 hr	0.34 ± 0.02	0.35 ± 0.02	0.38 ± 0.03	0.31 ± 0.05
at 48 hr	0.14 ± 0.02	0.15 ± 0.02	0.16 ± 0.02	0.14 ± 0.04
$T_{\frac{1}{2}}$ of DDS (hr)	18 ± 1	20 ± 1	20 ± 1	19 ± 2
$T_{\frac{1}{2}}$ of MADDS (hr)	19 ± 1	22 ± 1[b]	22 ± 1	22 ± 3

[a]Values are means ± SE.

[b]Significantly longer ($P < 0.05$) than the mean of the responders.

To assess possible differences between these patients and other populations we had studied previously, we examined the various correlations shown in Table 7. As in previous populations, we found that acetylation of SMZ in plasma and urine (line 1), and acetylation of SMZ and DDS in plasma (line 2) were positively related; that acetylation of DDS and the $T_{\frac{1}{2}}$ of DDS (line 3) were unrelated; and, that the $T_{\frac{1}{2}}$ of DDS and MADDS (line 4) were positively related. In these respects, the current Costa Rican groups are identical to the groups of Americans, Africans, Filipinos and South Indians we studied earlier (8,9,10,11).

Table 7

CORRELATION COEFFICIENTS OF VARIOUS METABOLIC PARAMETERS IN COSTA RICAN PATIENTS

Correlation Examined	Responders	Non-Responders		
		Total	DDS-Resistant	DDS-Sensitive
Acetylation of SMZ: plasma vs. urine	0.802	0.974	0.967	0.992
Acetylation in plasma: SMZ vs. DDS	0.812	0.926	0.943	0.893
Acetylation of DDS vs. $T_{\frac{1}{2}}$ of DDS	0.296[a]	0.431[a]	0.429[a]	0.460[a]
$T_{\frac{1}{2}}$: DDS vs. MADDS	0.982	0.954	0.969	0.983

[a]Correlation not significant; all others $P < 0.01$.

The mean $T_{\frac{1}{2}}$ values of 18 to 20 hr for DDS in all Costa Rican groups were nearly identical to the mean value of 21 hr found earlier in the DDS-resistant patients of varied racial and ethnic origins (3). They are also significantly shorter than the mean $T_{\frac{1}{2}}$ values of 28 to 29 hr for the African, Filipino and South Indian reference groups previously reported. The reasons for these differences in clearance are not apparent at this time.

In conclusion, this comparative study of the characteristics of acetylation and DDS disposition in non-responding and responding Costa Rican patients indicates that lack of response or DDS resistance is unrelated to any of the metabolic parameters we have measured.

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